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APPLICATION OF THE PURE-LINE CONCEPT TO BACTERIA *

L. J. COLE AND W. H. WRIGHT

From the Agricultural Experiment Station of the University of Wisconsin, Madison

In presenting this subject to geneticists we realize that much will be accepted by most of them, on the basis of their experience with higher forms of life, as more or less platitudinous. Nevertheless, since the facts with respect to bacteria may not be generally known to geneticists, and since the point of view which we shall present appears to be novel, or at least unappreciated, by most bacteriologists, we feel that a review and an interpretation of the facts in the light of present-day genetic advance will be of interest to both groups of investigators.

Variability in cultures was one of the most striking phenomena observed by the early investigators, and pleomorphism was commonly accepted by them. It was in fact the idea of early botanists, such as Nägeli and others, that "cocci could become bacilli, and bacilli, spirilla, as the chance of varying environment might dictate."[1] With the improvement of bacteriologic technic, however, the greater stability of bacterial forms became recognized; so that the definition of species and varieties, as well as of genera and higher groups, has been attempted after the manner employed in the classification of higher plants and animals. Nevertheless, the matter has always proved a vexatious one, owing to the great variability encountered, and as a result the classification of bacteria is still in a confused unsatisfactory state, and it is commonly very uncertain whether a particular form with which one may be working is the same or different from one which someone else has described. Much of the recent more careful work on variation has had as its object an improved classification, rather than the limits and causes of variation in themselves.

In their endeavor to delimit species bacteriologists have attempted to find the range in variation of their supposed species under a great

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[1] Winslow: The Systematic Relationships of the Coccaceae, with a Discussion of the Principles of Bacterial Classification, 1908.

variety of conditions, and because of the very small morphologic differences as compared with the physiologic plasticity, the latter has received the larger proportion of attention. In late years attempts have been made to apply the statistical method, particularly by Andrewes and Horder,[2] Bredemann,[3] Goodman,[4] Winslow,[1] Winslow and Walker,[5] Wolf,[6] and Buchanan and Truax.[7] Their studies have emphasized, what was already known from qualitative studies, that within recognized species there are distinct cultural races or varieties, each with its own characteristics and range of variability, and that these may exist side by side, independent of the environmental conditions. This belief is a distinct modification of the earlier view that one type could be transformed into the other directly by a change in the environment, or by selection.

As a result of his studies Winslow concludes that "bacterial types [species] should never be described on the strength of an examination of single individual strains; but only after a comparative study of the numerical frequency of each particular character in a considerable series of cultures." He lays down the following rules for classification: "First, each center of numerical frequency, marking a group of organisms varying about a distinct type in regard to a single definite property, may be recognized as a species. Second, those species which are bound together by the possession of a number of similar properties may be constituted as genera, and larger groups of genera, still characterized by some characters in common, may receive the rank of families or subfamilies." Winslow remarks that in the case of distinct bimodal curves "each mode may be considered a distinct type;" but he has apparently failed to consider the possibility that numerous distinct varying units, biotypes, may be masked in a unimodal curve.

TYPES AND CAUSES OF VARIATIONS

While, with respect to higher forms, there is coming to be a degree of unanimity as to the types of variation, even tho little may be known as to causes, the same can scarcely be said with respect to the bacteria. Altho a few recent investigators have attempted to bring order into this field, there has been a great deal of loose thinking and inconclusive reasoning on the subject, due, we feel sure, in most cases to a failure to appreciate the complexities and complications of the problem.

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[2] Lancet, 1906, 2, pp. 708, 775, 852.

[3] Centralbl. f. Bakteriol., Abt. 2, 1908, 22, p. 44.

[4] Jour. Infect. Dis., 1908, 5, p. 421.

[5] Ibid., 1909, 6, p. 90.

[6] Ztschr. indukt. Abstamm. u. Vererbungslehere, 1909, 2, p. 90.

[7] Jour. Infect. Dis., 1910, 7, p. 680.

The most common fact in the experience of the bacteriologist is that variation occurs in cultures in response to changes in the numerous factors in the environment—temperature, light, composition and reaction of the medium, and the like. There is not absolute agreement as to the heritability of variations produced in this manner, but one may state that the majority of bacteriologists consider them at least partially heritable. In fact, it is among bacteriologists and paleontologists, of all biologists, that a belief in the inheritance of acquired characters still has its strongest hold. In the case of the bacteria, the belief is based on the not uncommon observation that when a particular condition produces a change in a culture of organisms, and cultivation is continued a sufficient time under the condition, the variation may become so thoroughly fixed that there is no return to normal, even tho cultivation under the original conditions is resumed. In other words, it is believed that the change has been impressed on the organism by the conditions of the environment, and such changes are called impressed variations.

It has been noticed from time to time, on the other hand, that distinctly different types may occasionally be plated out from a given culture, altho there has been no observable difference in the environment. Since the publication by de Vries of his observations on mutation in higher plants, increasing attention has been given to this subject with respect to bacteria, and many cases of "mutations" have been described.

The views as to bacterial variation most commonly held are presented by Winslow.[1] In discussing variation he says: "Among asexual forms there is no swamping effect of amphimixis to bring exceptional variations back to the specific mean. With unicellular organisms again there is no bar to the persistence of acquired characters. Bacteria respond in many ways to the direct influence of environmental conditions; and each strain transmits to a degree the impress of its recent history. Selection, too, has exceptional opportunities to modify these quickly reproducing forms. The immense number of generations which may succeed each other in a short space of time makes boundary lines as shifting as they would become among the higher plants if a dozen geologic epochs were considered at once."

A classification of variations* is also presented, and later repeated in much the same form.[5] The following is compiled from both sources:

1. Observed differences in bacterial cultures, due to slight variations of the medium. These are considered as "not variations at all but temporary modifications of behavior due to the direct effect of existing conditions of cultivation;" this is not "real variation, altho in many cases it is mistaken for it."

2. There are 2 types of "true protoplasmic variations, which lead to characteristic differences in behavior under identical conditions." These are:

a. Variations "due to causes operating within the cells," arising "between the offspring of one original bacterium when growing under approximately the same conditions." In the absence of amphimixis this must be due to inequalities of nuclear division. Minute differences of this sort are "fluctuating variations," and others "well-marked sharply separated sports, or mutations." The former are common, the latter rare.

b. "Impressed variations" produced by the action of environmental conditions on a succession of bacterial generations. They are the impressions left upon an organism by its recent history; such as differences in virulence due to repeated animal inoculations, or the con-

* There has been much confusion in the use of the term variation. Some bacteriologists have not considered ordinary fluctuations as variations at all, while others, as noted, speak of heritable differences only, as true variations, inferring that other differences are not. On the other hand, variations have been considered technically by some biologists as referring only to nonheritable modifications, as quoted from Darwin by the Century dictionary. This confusion has arisen in part from the fact that some have thought of variation as an act or process of modification, while more commonly we speak of any existing differences in the material considered, as variations. Confusion again arises from the fact that there may be variation of different orders, as for example, between individuals of the same clone, between different clones in the same culture, between different cultures, or in fact between any groups. We may also have variation of the same group at different times.

Biologists are coming very generally to consider any measurable differences as variations, but it is extremely important that the nature of the material being considered should be clearly defined. It is often difficult to do this. Thus intraclonal variation would probably ordinarily be understood to mean variation of the individuals composing a single clone; it might also be applied, however, to the differences between separate cultures derived from the same clone. Similarly intraclonal selection might be based on the selection of individual cells of a clone, or on the selection of separate colonies, or simply on the selection of ordinary transfers or mass cultures made from a given clone. There are other terms which may prove useful, but in each case it will be necessary to define clearly just what is meant. Thus interclonal variation would mean variation in different clones. These might, however, be mixed in the same culture, or be in separate pure cultures; and again interclonal selection would refer to the ordinary selection of clones from a mixed culture, or might mean selection from different cultures, each representing a pure clone. The terms intracultural and intercultural, referring to variation or selection, are descriptive of definite conditions or processes, but are less specific than the other terms suggested.

It should be noted that in the foregoing discussion of definitions, no mention has been made as to whether the variations concerned are heritable or nonheritable. This must be determined for each case. It is customary now to speak of nonheritable variations as fluctuations, fluctuating variations or modifications, and of heritable variations as such or as mutations. There is no fundamental biologic difference between what Jordan[8] terms environmental modifications and latent characteristics; both fall in the class that we have designated fluctuations or modifications.

[8] Proc. Nat. Acad. Sc., 1915, 1, p. 160.

verse adaptations of pathogenic forms to culture media. They may be due to direct action between the protoplasm and the environment (as in the example just given) or to selective force acting on fluctuating variations, presumably of the type mentioned under 2a.

Before discussing further the types of variation which probably occur, it will probably be best to outline briefly the pure-line concept and to discuss the results of certain investigators in its light.

THE PURE-LINE CONCEPT

The idea of biologic groups, such as species, or even smaller units, especially of nonconjugating (vegetatively propagating) or self-fertilizing forms, being in reality complex populations of a number of independent hereditary lines, is so familiar to biologists that it scarcely needs exposition.

A pure line is defined by Johannsen[9] as "the descendants from one single homozygotic organism, exclusively propagating by self-fertilization;" but for our purposes in the case of bacteria, in which reproduction is asexual, it may be taken as the descendants from any single cell. (The term clone is coming to be largely used by biologists to designate the aggregate line of descendants from a single individual when reproduction is vegetative or asexual. While the distinction is preferable on genetic grounds, it has seemed best not to employ the word in the present instance, since the general principle we are discussing is the pure-line concept whether applied to strictly pure lines, or to clones.) If a culture were started from 2 independent single organisms, successive transfers, unless 1 should be lost, would consist of 2 pure lines carried down side by side. While it cannot be accepted without proof, it seems fair to assume that if heredity is to be credited, the descendants of one of the cells should in the aggregate resemble more closely one another than they would the descendants of the other original cell, provided there was any hereditary difference in those two. There would thus be not only 2 pure lines of descendants, but 2 distinct genotypes, or hereditary complexes. If the hereditary composition of the two original cells were exactly the same, there would be but a single genotype, altho according to Johannsen's usage, there would still be represented 2 pure lines. In common usage, however, the terms pure line, genotype, and biotype have come to be used interchangeably to designate groups of organisms which are assumed to have exactly the same hereditary complexes, whether or not they are known to be descended from a single common ancestor.

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[9] Am. Nat., 1911, 45, p. 129.

The epoch-making researches of Johannsen[9] and of Jennings[10] have demonstrated the existence of distinct hereditary lines in self-fertilizing plants and in the protozoa, while subsequent work has shown that many of the phenomena of cereal-breeding may be referred to the same cause. An ordinary field of wheat, for example, tho it may all be considered a single variety, in reality consists of a mixture of hereditary types, or biotypes, each of which has its characteristic range of variation, and its own definite mode of response to environmental changes. In other words, the individuals of a given biotype, tho of the same hereditary nature, may exhibit a considerable range of variation in response to even slight differences in the environment. In this way, while the ranges of variation of two biotypes would not be entirely coincident, they might overlap to a considerable extent, and it would consequently be impossible to tell from the character of a single individual in this overlapping region whether it belonged to the one biotype or to the other. The only way to determine the matter would be to consider statistically a considerable number of descendants from the selected individual, for these should give the range of variation and mode characteristic of the type to which they belong.

In connection with the foregoing interpretation it is assumed that the environment does not ordinarily produce any change in the hereditary qualities of a biotype; so that variations produced by changed environmental conditions should have no permanent effect, the type returning to normal when normal conditions are restored. In recent years abundant evidence has been accumulated to prove the general truth of this assertion with respect to many animals and plants, and we shall see that it serves to explain also many of the phenomena observed in connection with bacteria. In practical cereal-breeding it has been found that often a single desirable individual may be selected which will breed true to its desirable qualities, so that all the desired results of selection are accomplished at a single stroke, and all that is necessary is to multiply the form by further propagation. This is the pure-line method of selection, in which the breeding value of an individual is judged on the basis of the character of its progeny.

We know, however, that mass selection is also productive of results, tho progress is much slower, and is often not permanent unless the selection is strictly continued. In mass selection, instead of separating out different biotypes at once, a number of lines are carried along at each selection, and as the more desirable ones predominate, and the less desirable drop out, progress is made. With this method it is very difficult to eliminate the undesirable biotypes entirely, and this explains why, when selection is relaxed, they may again rise to ascendancy. While, therefore, there has been an apparent change of the culture, or crop, as a whole, it has been apparent only, and not hereditary, except in so far as certain biotypes may have been dropped out. It must be borne in mind that changed environment or selection does not alter the hereditary qualities of the individual lines, tho it may eliminate or temporarily suppress some of them to the advantage of others.

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[10] Am. Nat., 1909, 43, p. 321; 1911, 45, p. 79.

Many bacteriologists have isolated and investigated strains of organisms which have remained true to characteristics different from those of the culture from which they were taken, yet few have grasped the full significance of the pure-line viewpoint as just outlined. Among those who have come most near to it may be mentioned Wolf[6] and Buchanan and Truax.[7] We shall now select a few cases to show how the pure-line concept furnishes an adequate explanation for facts not altogether harmonious on other views.*

Dyar,[11] who made a careful study of Bacillus lactis-erythrogenes, poured plates of his organism and made cultures from isolated colonies, assuming that each consisted of the descendants from a single cell. Studies of the variability of the cultures obtained showed that most of them tended to return to the mean of the original culture. These results were taken to indicate the comparative permanency of species and races. It must be pointed out, however, that the presumption that single colonies are always, or perhaps even largely, the descendants of single cells, no matter how great the dilution, must be regarded with suspicion, and that the burden of proof lies on explanations based on results obtained by this method. Support of this contention is to be found in Dyar's own results later on, as he himself admits, and it is confirmed further by the recent investigations of Breed[12] on lactic-acid organisms (see also Drew[13]).

From certain of his cultures Dyar obtained a form which produced a crusty wrinkled growth in contrast to the normal smooth form. It seemed to appear suddenly, and he accordingly considered it a "sport or discontinuous variation." An agar-plate dilution was made from this crusted form and cultures taken from 125 isolated colonies; of these all but 3 came true to the new type, tho some cultures showed soft borders. The three were entirely of the normal soft form. The general result of succeeding tests was the same; that is, there always appeared to be a tendency toward a reversion to the normal type from the crusted form, but cultures from these reversions remained nearly true to type, only 1 culture from 480 isolated colonies showing again the crusted form. The explanation of this apparent mutation from normal, reversion to normal, and mutation again to the crusted form, and also to a new intermediate·type, is hinted at by Dyar himself. He says (p. 336): "It is conceivable that when the skinny growth of the wrinkly form is agitated in sterile water preparatory to making plates, that on account of its property of coherence, instead of becoming separated into single cells, certain of which are subsequently to develop colonies, the smallest portions really consist of masses of cells which might entangle a few cells of the smooth form, if this were originally present as a contamination. Thus the resulting colonies might be impure and the apparent phenomena of reversion be due to a separation of this mixture. Now the phenomena described above, of the soft borders to the wrinkly agar culture and colonies, are in favor of this view, and it is also observed that the growth when shaken up in water disintegrated with great difficulty." But as to the soft form, "a softer growth is scarcely to be imagined," and "in the hanging drop, the cells are seen singly, or, rarely, in pairs or short chains. It is, therefore, highly improbable that these should form masses and entangle a few of the wrinkly form."

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*Since the writing of this paper there has come to our attention an extensive work by Toenniessen, "Ueber Vererbung und Variabilität bei Bakterien" (Biol. Centralbl., 1915, 35, p. 281), which cannot be reviewed at this time.

[11] Ann. New York Acad. Sc., 1895, 8, p. 322.

[12] Centralbl. f. Bakteriol., Abt. 2, 1911, 30, p. 337.

[13] Bull. New York Agr. Exper. Sta., No. 373, 1914.

Cultures of the same organism were exposed to high temperature (37.5 C.), and as is common in chromogenic bacteria, they tended to lose color and grow less vigorously. One culture became white around the edge; another grew paler and finally became spotted. Cultures from the white and yellow colonies showed distinct differences. Here again Dyar hit on an explanation which coincides with the pure-line concept. He concludes again that his cultures were mixtures, and that the white form was less injuriously affected by increased temperature; so that "the apparent effect of the temperature in producing a white form in the first instance may have really been due to a process of selection, the white [form] growing the faster and tending to supplant the other." "The variations toward a white form probably tend to become eliminated under natural conditions where the yellow form doubtless grows best. . . . It may be that this is, at least in great part, the true explanation of the production of the colorless races of chromogenic bacteria, which has been quoted as proof of the 'transmission of acquired characters.'"

It seems fair to conclude that Dyar was in reality working with a number of biotypes. The important question is, were these biotypes all represented in the original culture, or did they arise spontaneously or in response to environmental influences? Dyar's work does not permit of a conclusive decision on this point, tho he attempted to make his conditions as uniform as possible.

Goodman[4] developed 2 distinct forms of the diphtheria organism from 1 strain. One gave high acidity in dextrose broth and the other finally produced an alkaline reaction. The author concludes that there are no true distinguishing marks between the different forms, since the zymogenic power can be altered at will by artificial selection. He does not consider the possibility of his selections having separated 2 strains present in his original culture. This explanation must be considered, however, since we have seen from Dyar's results that the plating method is unreliable for separating single organisms.

Bredemann[3] studied statistically 27 cultures of Bacillus asterosporus isolated from soil samples from all parts of the world. Frequency curves showed distinct modes which remained permanent for 3 years, yet there was no doubt that all were very closely related, and if they had all been put together the resulting mixed culture would no doubt have given the impression of considerable uniformity, unless means were taken to separate the different lines again.

Winslow and Walker[5] in a study of 2 strains of the paratyphoid bacillus, found that in spite of selection the frequency curves of the two remained distinct as to range and mode, altho the ranges overlapped. The difference between their results and Goodman's may be attributed to the fact that their two cultures, A and B, were true single biotypes to begin with, whereas his culture was mixed.

Buchanan and Truax[7] carried cultures of Streptococcus lacticus through 23 transfers, and attempted by selection to secure high- and low-acid-producing strains. Their efforts were unsuccessful. They decided that their failure was due to the fact that they were working with a pure line, within which selection was ineffective, and concluded that "the simplest method of securing high- and low-acid races of S. lacticus would be to select from a great number of sources in an effort to secure such races already established."

Rettger and Sherrick[14] were able by selection to secure divergence in color-production in certain chromogenic bacteria. They selected from portions of cultures which were the most or the least pigmented. As they themselves point out, their results may be due to a selection of already existing variations, rather than a production of variation by selection.

Hirschberg[15] reports negative results in an attempt to select for differences of shape in Bacillus coli.

Beijerinck[16] made extensive studies of variation and the effects of selection in bacteria, from which he drew far-reaching conclusions. In one case he reports profound physiologic modification of certain soil organisms which change nitrites to nitrates in the soil, and which thrive only in solutions of organic salts. By adding organic matter gradually in increasing amounts to the culture media, the organisms ultimately came to grow well in ordinary broth and lost entirely their power to grow in inorganic salt solution, or to oxidize nitrites to nitrates. From the phosphorescent organism Photobacter splendidum, isolated from the waters of the North Sea, cultures were obtained which lacked luminosity entirely, and others which were intermediate with respect to the light-producing function. The variations appeared in some cases to be reversible; luminous forms, for example, being obtained again from the dark mutant. These various changes Beijerinck classifies as mutations, submutations, modifications, and atavism, and discusses them in their relation to the "genenhypothese." One feels, however, that for genetic interpretation a much clearer analysis of the different forms and their origin is necessary.

The foregoing demonstrates that most bacterial cultures are mixed populations of a larger or smaller number of distinct hereditary forms or lines. Different cultural methods or other environmental changes act as a sieve through which the multiplex populations are sifted. Under a condition unfavorable to them certain biotypes become lost, or remain in a minority, while those forms flourish which are adapted to that particular condition. Upon a return to normal the conditions are reversed, and the formerly suppressed types again assert themselves, provided the previous conditions have not been so severe as to kill them off entirely. It is just this chance which accounts for the inconstancy and lack of conformity of results of selection on mass cultures. Artificial selection under constant conditions would act in essentially the same way.

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[14] Jour. Med. Research, 1911, 24, p. 265.

[15] Biometrika, 1913, 9, p. 331.

[16] Folia Microbiologica, 1914, 3, p. 91; 1915, 4, p. 15.

Many of the striking phenomena of bacteriology may be explained on this basis, particularly adaptations in bacterial cultures. As a special example may be mentioned the findings of Rosenow that streptococci isolated from a particular location or organ show a selective action for the same organ when introduced into a new host. Thus streptococcus cultures from stomach ulcers tend to produce stomach ulcers. Our explanation would be that the stomach environment, possibly the specific secretions, have tended to bring into predominance streptococcus biotypes especially adapted to that environment, and when they are inoculated into another animal, being the predominating forms they naturally grow most readily in the environment to which they are adapted, and in turn produce stomach ulcers in the new host.

Similarly, as Rettger and Sherrick have pointed out in speaking of the variations in virulence of pathogenic organisms, animal passage is "a mode of eliminating the avirulent individuals, and of encouraging the production of a pure pathogenic type."

THE EVIDENCE FOR MUTATIONS IN BACTERIA

We have seen that in the case of most of the results which have been put forward as examples of true hereditary mutations in bacteria the evidence of their really being such is inconclusive. They may as well be explained as the isolation of biotypes which were present, but not in evidence, in the original culture. The persistence of bacteria with little if any growth under unfavorable conditions is well known, and is illustrated by our own experiments on the persistence of lactic-acid organisms in highly diluted milk, in which the organisms remained in an apparently quiescent condition for long periods. The criterion of a mutation is that it must appear in a known pure line. It need not be of great magnitude or discontinuous in the sense that its variations may not overlap those of the parent form; but it must show a distinct racial modality of its own. Few experiments which meet these conditions have been performed, and they can be briefly reviewed.

Hansen[17] and Barber[18] each worked on cultures from isolated single cells. They used both yeast and bacteria. Hansen was able to develop physiologically typical cultures of bottom-fermentation yeast from top yeast, by cultivation of the progeny of single cells at a low temperature. He maintains that any variation which shows constancy must be taken as a mutation. His results with bacteria were somewhat similar. Barber obtained races of yeast showing morphologic differences by selection among the descendants from a single cell, but he estimated that the cells of the type which produced the new race occurred on an average of less than 1 in 5,000. From a culture of Bacillus coli-communis started from a single isolated cell he was able to produce a new race producing longer chains twice in 190 selections. The new race, while variable, showed constant difference from the parent form, and altho selection was practiced from each extreme of the new race, "there was neither accentuation of the peculiarity nor return to the original type." These would appear to have been true mutations and to have occurred spontaneously, as far as we can tell.

Wolf,[6] on the other hand, believes that he was able to induce mutations by change in environmental conditions, especially by the addition of certain poisons, such as corrosive sublimate, potassium bichromate, etc., to the culture media. Objection may be raised to Wolf's results on the ground that he depended upon the isolation of colonies from plate dilutions, yet it must be confessed he took extreme precautions. In the case of Bacillus prodigiosus he was apparently able to produce occasional mutations both of a lighter and of a darker shade of color than was to be observed in the original culture.

Jordan[8] recently reported what appears to be a perfectly clear case of mutation in Bacillus coli, which occurred under adequately controlled experimental conditions. A single cell was isolated by the Barber method from a freshly cultivated feces culture, and later from this 2 substrains were started from isolated single cells. These were carried through more than 500 transfers without showing any permanent change in their various characteristics, except that in one line a form appeared having the power of saccharose-fermentation. "This quality appeared in the seventh transfer on sodium-chlorid agar. It was not manifested by all the cells of the culture, but at the time of examination the saccharose-fermenting cells were greatly in the majority. On continuing the transfers they became the sole type found in the cultures, the nonsaccharose type disappearing altogether. . . . The newly acquired fermenting property has remained permanent throughout a series of over 500 test-tube generations (forty-eight-hour transfers), and is shown both by the cultures on sodium-chlorid media and by the strains transferred immediately on the aquisition of this property to ordinary nutrient agar and grown side by side with the two parent strains." In this case as in Barber's the mutation appears to have been spontaneous rather than induced.

PRACTICAL APPLICATIONS

If the interpretation here presented is established by future investigations, it will have a profound influence on practical bacteriologic methods, as the findings of Hansen, cited in the foregoing, have already had in the brewing industry. It is now customary to go beyond species lines, and to employ particular strains of bacteria for particular purposes, as, for example, in the manufacture of butter and cheese, while a particularly striking example is the use of a certain strain of the diphtheria organism, originally isolated by Park, for the manufacture of diphtheria antitoxin.

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[17] Compt. rend des travaux du Lab. de Carlsberg, 1900, 5, p. 39. Abstracted in Centralbl. f. Bakteriol., Abt. 2, 1901, 7, p. 439. Ibid., 1905, 12, p. 353.

[18] Sc. Bull. Kansas Univ., 1907, 4, p. 3.

It has been very generally assumed that most of such strains have been produced by a modification of the typical organism, and that they are only relatively permanent, continual selection being necessary to keep them constant. Others, however, such as the diphtheria culture mentioned, have shown remarkable constancy under all conditions. In all probability the degree of constancy is directly correlated with the degree to which the strain approaches a single biotype or pure hereditary line, instead of being a mixture.

Should it be found that strains derived from single organisms show the high degree of constancy expected, the method of isolating lines in this way would be of great help in establishing cultures of any desired degree of virulence or other quality. Even then it would be necessary, of course, to guard against contamination, and also to be on the lookout for possible mutations which might occur within the culture. The procedure on the whole, however, would be more direct and more certain than the colony method of isolation.

CONCLUSIONS

There has been much confusion regarding variation in bacteria.

Most of the variation observed in ordinary cultures is due to the selection, more or less complete, by the investigator or by the environment, of particular pre-existent biotypes.

If the process of selection is complete, so that certain biotypes are completely lost, the culture will not return to normal type, even when placed again under normal conditions. Such a change is apt to be mistaken for a true hereditary mutation.

When the process of selection is incomplete certain biotypes are raised into predominance and others are suppressed, altho they may remain alive in the culture. In this case the return to normal conditions brings out the dormant types adapted to those conditions and the culture resumes its original characteristics. Such changes have commonly been referred to as modifications, tho they differ only in degree from those described in the foregoing.

The descendants of a single cell constitute a pure line, or, more strictly, clone, and on such material, altho there is variation, selection is in no way effective unless actual hereditary mutations occur and are separated out.

Mutations apparently do occur in pure lines, both spontaneously and in response to certain environmental changes. Such mutations give rise to new races, or biotypes, which vary about modes of their own, and within which selection is ineffective.

Mutations may be of a magnitude to be readily appreciated, or they may be so small as to be determinable only by statistical methods.

The biotypes which make up the ordinary bacterial culture have undoubtedly arisen in times past as distinct mutations, and have lived on side by side, retaining their individuality because of the absence of amphimixis.

It is only by the most refined methods that the foregoing kinds of variations can be recognized and differentiated.